



Denitrification in a seashore sandy deposit influenced by groundwater discharge

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Abstract. The chemical compositions of ground water and organic matter in sediments were investigated at a sandy shore of Tokyo Bay, Japan to determine the fate of ground water NO₃⁻. On the basis of Cl⁻ distribution in ground water, the beach was classified into freshwater (FR)-, transition (TR)-, and seawater (SW)-zones from the land toward the shoreline. The NO₃⁻ and N₂O did not behave conservatively with respect to Cl⁻ during subsurface mixing of freshwater and seawater, suggesting NO₃⁻ consumption and N₂O production in the TR-zone. Absence of beach vegetation indicated that NO₃⁻ assimilation by higher plants was not as important as NO₃⁻ sink. Low NH₄⁺ concentrations in ground water revealed little reduction of NO₃⁻ to NH₄⁺. These facts implied that microbial denitrification and assimilation were the likely sinks for ground water NO₃⁻. The potential activity and number of denitrifiers in water-saturated sediment were highest in the low-chlorinity part of the TR-zone. The location of the highest potential denitrification activity (DN-zone) overlapped with that of the highest NO₃⁻ concentration. The C/N ratio and carbon isotope ratio (δ¹³C) of organic matter in sediment (< 100 -μm) varied from 12.0 to 22.5 and from -22.5 to -25.5‰, respectively. The δ¹³C value was inversely related to the C/N ratio ($r^2 = 0.968$, $n = 11$), which was explained by the mixing of organic matters of terrestrial and marine origins. In the DN-zone, the fine sediments were rich in organic matters with high C/N ratios and low δ¹³C values, implying that dissolved organic matters of terrestrial origin might have been immobilized under slightly saline conditions. A concurrent supply of NO₃⁻ and organic matter to the TR-zone by ground water discharge probably generates favorable conditions for denitrifiers. Ground water NO₃⁻ discharged to the beach is thus partially denitrified and fixed as microbial biomass before it enters the sea. Further studies are necessary to determine the relative contribution of these processes for NO₃⁻ removal.

Introduction

The discharge of ground water often provides an influx of soluble compounds to surface aquatic systems. Nutrients in discharged ground water are important for the element budget of lakes (Lee et al. 1980; Frape and Patterson 1981; Brock et al. 1982). They also play key roles in the nutrient dynamics of some coastal aquatic

systems (Valiela et al. 1978; Johannes 1980; Capone and Bautista 1985; Zimmermann et al. 1985; Slater and Capone 1987). Among various solutes in ground water, NO_3^- is a necessary nitrogen source in oligotrophic environments such as tropical coral ecosystems (D'Elia et al. 1981; Lewis 1987; Matson 1993).

High nitrate concentration in ground water is widely recognized (e.g., Power and Schepers (1989) and Strebel et al. (1989)) and is of great concern in regard to human health (Comly 1945; Virgil et al. 1965). The NO_3^- contamination of shallow aquifers is often related to anthropogenic impacts such as fertilizer application, sewage infiltration, and waste landfill. Recent studies indicate that ground water NO_3^- may enhance the productivity of coastal seas (Sewell 1982; Giblin and Gaines 1990; Simmons 1992; Paerl 1997) and sometimes causes the appearance of a hypoxic zone along the coast (Goolsby 2000). Nitrate inputs thus influence marine ecosystems not only through river runoff but also through ground water discharge.

Studies of nitrogen dynamics in various types of land-sea boundaries, such as sediments in tidal flats, salt marshes, estuaries, and coastal seas, suggest sedimental denitrification to be a hot spot in the biogeochemical nitrogen cycle (Rosswall 1983; Christensen et al. 1987; Seitzinger 1988). Although past studies emphasized a nitrogen flux between water and the benthic sediment, the nitrogen dynamics in sandy beaches remain poorly characterized. This is because microorganisms are probably less active in beach deposits than in benthic sediments due to the typically low abundance of organic matter in a sandy beach (Brown and MacLachlen 1990). Thus, discharge of ground water to a sandy beach, which may be a significant nitrogen source for a coastal environment, has received little attention.

Nitrate reduction in assimilatory and dissimilatory processes in a land-sea boundary could act as a biological filter for NO_3^- discharged into the boundary. In particular, denitrification is a potential sink for the nitrogen added to the beach. Several environmental variables, such as oxidation-reduction condition and substrate availability, control denitrification in the field. We observed the spatial variations of these variables in a sediment-ground water system at a sandy beach in Tokyo Bay. We also measured the number and potential activity of denitrifiers in the beach sediment. The goal was to examine whether denitrification could take place in the sandy beach sediment.

Materials and methods

Site description

The study site was located in Sanuki Town in Futtsu City, Chiba Prefecture, Japan (Figure 1). It consisted of a natural sandy beach that had developed at the foot of a slope from an adjacent plateau. Arable fields, residences, and forests were typical land covers on the plateau. Between September 1 and 21, 1994, a 13-m-long soil core sample was taken near a private well site (Figure 1) by the OYO Corporation (Saitama Prefecture, Japan). The core sample showed that both unsaturated (0–3.5

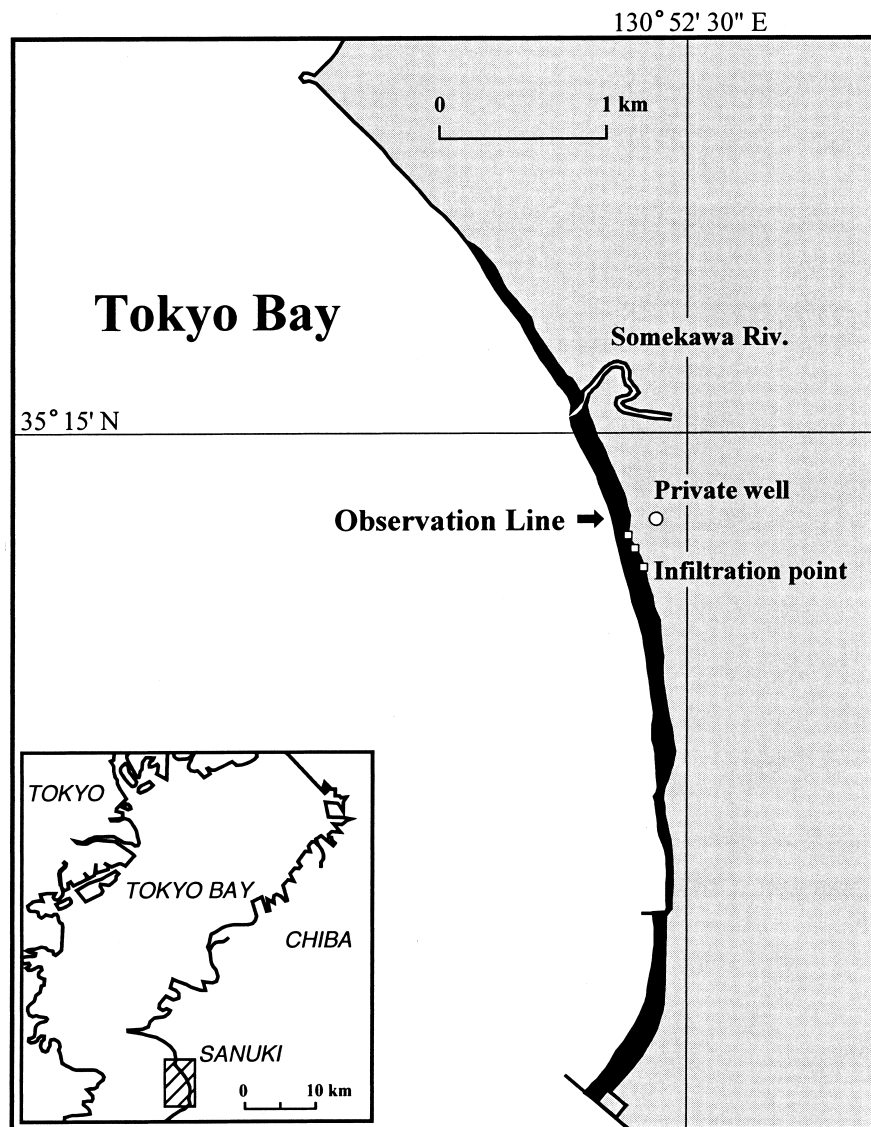


Figure 1. Study site. The black area indicates sandy deposits. The circle and squares present locations of a private well for household and infiltration points for domestic sewers, respectively.

m depth) and saturated (3.5–5.5 m depth) zones consisted of sand. The saturated zone was underlain by an impermeable layer (5.5–13.0 m depth) mostly consisting of clay. The unconfined aquifer water of the plateau discharged into the sandy deposits over the impermeable clay layer.

On the sandy beach, we established a 50-m-long observation line that met the shoreline at a right angle for determining horizontal variation in the chemical com-

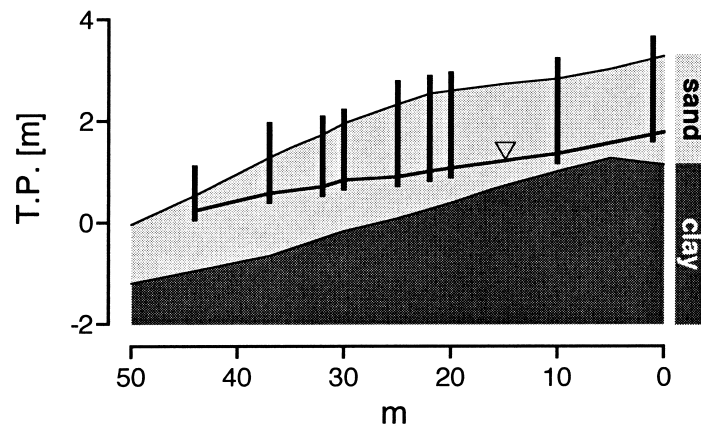


Figure 2. Cross section on September 20, 1994. 'T.P.' is an abbreviation of Tokyo Pail that is mean sea level of Tokyo Bay. Bold vertical bars show locations of the single-strainer wells. '▽' indicates water table.

position of beach ground water. The mean values of the coefficient of permeability and porosity of sands at several locations of the beach were $2.5 \times 10^{-2} \text{ cm s}^{-1}$ and 0.48, respectively. The ground water velocity toward the shoreline, observed by the salt tracer test, changed little throughout the year ($3.8 \pm 0.4 \text{ m d}^{-1}$; E. Sumi, unpublished data) and was independent of temporal variation in the location of the water table.

There were three infiltration pits for domestic wastewater along the landside fringe of the sandy beach (Figure 1). Our preliminary survey had indicated high NO_3^- concentrations in ground waters at the downstream of the filtration pits. Suzumura et al. (2000) mentioned that partial phosphate in ground water below our observation line might have originated from the wastewater. Although pit locations were out of our observation line, sewers would be an additional NO_3^- source to the ground water discharge.

Water and sediment collection

Single-strainer wells were prepared using bottom-capped PVC pipes (65 mm ID) and stainless steel screens (100- μm mesh) after Cherry et al. (1983). These wells allowed us to collect vertically composite ground waters 0–15 cm below the water table. Six to ten single-strainer wells were buried on the observation line at appropriate intervals to collect samples with variable salinity on September 20, 1994, July 11, 1995, July 3, 1996, and September 26, 1995 (Figure 2). Waters accumulating in the single-strainer wells contained fine sediments soon after installation. The turbid waters were removed using a hand-operated aspirator. Water samples for chemical analyses were collected on the next day.

A multiple-strainer well (Cherry et al. 1983) was used to collect waters along a depth profile. This well was made of a stainless-steel pipe with a sharpened bottom

(34 mm OD, 28 mm ID, 2 m long). The pipe was drilled at 10-cm intervals from the bottom to make side pores (1-mm) as strainers. Inner spaces between pairs of pores were segmented into compartments by disc-partitions made of silicone rubber. Each disc-partition was assembled by a stainless steel tube (1.6 mm OD) with a Leur fitting, so that water could be obtained from different compartments independently by using plastic syringes. This handmade probe-like well was inserted into the sand at 25 and 34 m from the land side of the beach on August 25, 1995.

On the next day of installation of single-wells, beach ground waters, water from an electric pump-well for a house located upstream of the observation line (Figure 1), and near-shore seawater were collected. Roped cylindrical buckets (50 mm OD, 300 mm long) were used to collect water samples from the single-strainer wells during ebb tide periods. The bucket was equipped with a metal disc and a rubber gasket at the bottom. This type of assembly allowed water to fill the sampler, but not leak from it. On September 21, 1994 and September 27, 1995, water samples for determining dissolved gas concentrations were taken before water collection for ion analyses. No measurement for dissolved gas was carried out in other observations. Water for O_2 concentration measurement was collected in a 100 -ml BOD glass bottle without bubbling and immediately fixed by the Winkler method. Water to measure N_2O concentration was collected in a 30-ml glass vial in triplicate. Each vial was then plugged by a butyl rubber stopper and an aluminum clip after 0.1 ml of saturated $HgCl_2$ had been added. On August 25, 1995, waters for determining vertical change in salinity and NO_3^- concentrations were collected using the multiple-strainer well.

During the installation of the single-strainer wells on July 11, 1995, September 26, 1995, and July 3, 1996, sediment 0–20 cm below the water table was taken at 5–6 points between 10 and 40 m from the landside fringe to determine the potential activity and number of denitrifiers. The particles in the turbid waters that had been evacuated from the single-strainer wells on July 11 and September 26, 1995 were used to characterize sedimental organic matter.

Chemical analyses

Dissolved O_2 concentration was determined by titration. Dissolved N_2O was analyzed on an ECD gas chromatograph (Shimadzu GC-8A) tagged by a purge-trap gas extraction device. The detection limit and reproducibility of the N_2O measurement were $0.01 \mu g N l^{-1}$ and $\pm 2\%$, respectively. The atmospheric equilibrium concentration of N_2O was calculated after Weiss and Price (1980) based on the *in situ* water temperature and a fixed N_2O mixing ratio, 310 ppm (v/v). Waters for analyses of ionic components were filtered through pre-combusted glass fiber filters (Whatman GF/F). The concentrations of NO_3^- , NO_2^- , and Cl^- in low-salinity waters were determined by ion chromatography. In high-salinity samples, NO_3^- and NO_2^- concentrations were measured by Wood et al. (1967), and Cl^- concentration was measured by titration. Concentration of NH_4^+ was measured following the method of Solorzano (1969). Temperature and salinity were determined using elec-

tric conductivity meters (Wissenschaftlich-Technische Werkstaetten Konduktometer LF 191).

Denitrification activity

Denitrification activity within beach sediments was determined by the acetylene inhibition technique (Yoshinari and Knowles 1976). The sediments collected in plastic bags were homogenized, and 50-g portions were weighed in 60-ml glass vials in duplicate or triplicates. Five ml of KNO_3 solution (7 mg N l^{-1}) was added to the vials, which were subsequently plugged by butyl rubber septa under a N_2 atmosphere. One ml of water-washed acetylene was injected into the vial through a septum. The vials were incubated at $25 \pm 1^\circ\text{C}$ in darkness for 3 hours. At the end of incubation, 1 ml of saturated HgCl_2 solution was added to each vial to cease denitrification activity. Preliminary experiments confirmed a time-dependent linear increase of N_2O concentration in the headspace of the vial for the first six hours. All the above procedures were carried out within 24 hours after field sampling.

Potential denitrification rate per gram of dry sediment was calculated from N_2O accumulation in the vial. Concentration of N_2O in the headspace was measured by ECD gas chromatography after the vial was vigorously hand-shaken to achieve gas-liquid equilibrium. Since the headspace N_2O content represents the product of the gaseous concentration and the volume of the headspace, the headspace volume in each vial was measured as the weight of the water required to fill it. The aqueous N_2O fraction was calculated by multiplying the concentration of dissolved N_2O with the volume of water. The dissolved N_2O concentration was estimated from the headspace concentration and the equilibrium constant at 25°C . The water volume in each vial was calculated by totaling the volume of original pore water in the sediment sample and the volume of the KNO_3 solution added. The present method had the detection limit of $0.1 \mu\text{g N kg}^{-1} \text{ d}^{-1}$ and yielded a minimal potential denitrification rate because of static incubation.

Enumeration of denitrifiers

The most-probable-number (MPN) method (APHA-AWWA-WPCF 1980) was used to count denitrifying bacteria in sediments taken in sterilized plastic containers at the locations where the samples for denitrification activity measurements were collected on July 11 and September 26, 1995. A few grams of sediments were weighed and dispersed in a 200-ml sterile physiological salt solution (NaCl ; 0.85 g l^{-1}). After two minutes sonication (65 W, 36 kHz) of the suspension, the supernatant was used as an inoculum source. Ten-fold serial dilutions of the inoculum source were prepared in the sterile physiological salt solution, and then 1-ml portions were transferred into five replicate test tubes. The test tubes containing the Giltay medium (pH at 7.0–7.2) and the Dahrum tubes had been previously sterilized by autoclaving. The medium consisted of KNO_3 , 2.0 g; asparagine, 2.0 g; BTB solution (1%, w/v), 10 ml; sodium citrate, 17.0 g; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 2.0 g; $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.1 g; KH_2PO_4 , 2.0 g; and $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 0.4 g, in 1 liter of distilled water. The inocu-

lated tubes were incubated at 30 °C for 14 days. Results were reported in MPN per dry weight of sediment.

Isotopic characterization of organic matter in fine sediment

Fine sediment was collected on a pre-combusted glass fiber filter (Whatman GF/F) by filtering the turbid water that had been obtained during well installation. The fine sediments were treated with 1 mol l⁻¹ HCl and washed with de-ionized water to eliminate any carbonates and salts. The samples were dried at 60 °C in an oven and homogenized in a mortar. Contents of organic carbon and nitrogen and isotope ratios of organic carbon in the fine sediments were determined by the sealed-tube combustion method (Minagawa et al. 1994) with slight modification. A sample of 50–100 mg was weighed and mixed with an equal amount of CuO powder. The mixture was put into a pre-combusted quartz ampoule (3/8" OD, 300 mm long), and 1.0 g of CuO wires, 0.5 g of Cu wires, and a piece of Ag ribbon were added. The ampoules were evacuated and flame-sealed and placed in an oven at 850 °C for 2 hours followed by gradual cooling. Sedimentary organic carbon and nitrogen in samples were converted stoichiometrically into CO₂ and N₂, respectively. The CO₂ and N₂ were separated by cryogenic means while their volume was measured manometrically. The carbon isotope ratio of CO₂ measured on a mass spectrometer (Finnigan MAT 252) was expressed as the *per mil* deviation from a reference material: $\delta^{13}\text{C}, \text{‰} = (\text{R}_{\text{sample}}/\text{R}_{\text{reference}} - 1) \times 1000$, where R denotes ¹³C/¹²C and V-PDB is the reference material. The reproducibility of the measurement was better than ± 0.1‰.

Results and discussion

Mixing of fresh and sea waters

Horizontal variation in Cl⁻ concentrations of the beach ground water confirms that freshwater mixes with seawater in the sand matrix (Figure 3). The Cl⁻ levels were low (< 0.1 g l⁻¹) in the 1–20 m region on September 27, 1994 and July 4, 1996. The low-chlorinity region stretched to 25 and 33 m on September 27, 1995 and July 12, 1995, respectively. An elevated discharge of fresh ground water or an *in situ* recharge by rainfall possibly caused such seaward expansion of the low-chlorinity region. The Cl⁻ concentrations at the nearest point to the shoreline approached the seawater level (~ 18 g l⁻¹). The Cl⁻ gradient categorized the beach subsurface environment into three compartments: a freshwater zone (FW-Z), where ground water with low chlorinity (< 0.1 g l⁻¹) existed, a sea-water zone (SW-Z), where sea water intruded into the beach sediments, and a transition zone (TR-Z), where ground water had variable chlorinity ranging between those of FW-Z and SW-Z.

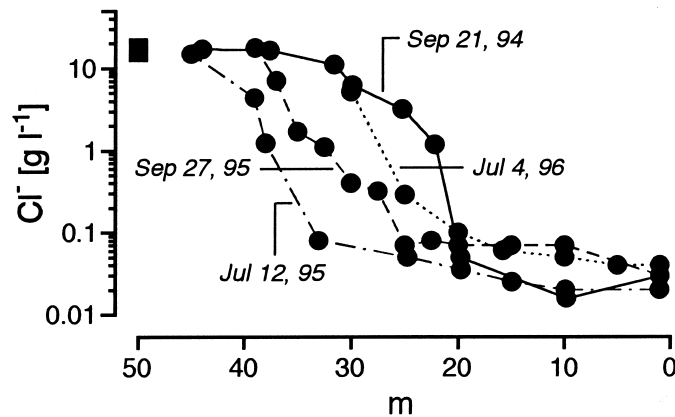


Figure 3. Cl^- concentration in ground water [●] and seawater [■] along the observation line.

O_2 concentration

The dissolved O_2 concentrations in the ground waters were below atmospheric equilibria at 1- and 10-m points (Figure 4a, 4b). Ground water from the municipal well located upstream of the observation line showed lower O_2 concentration (1.7–2.0 mg l^{-1} , 19–23% saturation) than that in the 1–10 m region owing to soil respiration in the catchment. On September 27, 1995, the ground waters at 15 and 20 m were over-saturated with respect to atmospheric O_2 (Figure 4b). These observations are probably due to on-site recharge by oxygenated rainwater in advance or the sampling. Seaward shifts of FW-Z and TR-Z on September 27, 1995 (Figure 4b), in comparison with September 21, 1994 (Figure 4a), support this view. The TR-Z ground water was in equilibrium with atmospheric O_2 . The rapid O_2 exchange between water and atmosphere due to wave oscillation accounts for the equilibrium O_2 concentrations in SW-Z ground water and near-shore seawater. Thus, ground water in TR-Z through SW-Z was generally aerated.

NO_3^- and N_2O dynamics in TR-Z

Concentrations of NO_3^- in ground water decreased from TR-Z to SW-Z on September 21, 1994 and September 27, 1995 (Figure 5), as on other dates (data not shown). The highest NO_3^- concentration of FW-Z ground water (8.9 mg N l^{-1}) exceeded that of the private well-located upstream water (6.0 mg N l^{-1}). According to our preliminary survey, the NO_3^- concentration of ground water located 15 m downstream of the infiltration pit was 11.8 mg N l^{-1} , which indicated that FW-Z ground water might contain NO_3^- derived from sewage that had infiltrated onto the beach. Nevertheless, source elucidation of NO_3^- is unimportant in relation to the total input to the TR-Z. Concentrations of NO_2^- and NH_4^+ were very low ($< 0.02 \text{ mg N l}^{-1}$) in all observations.

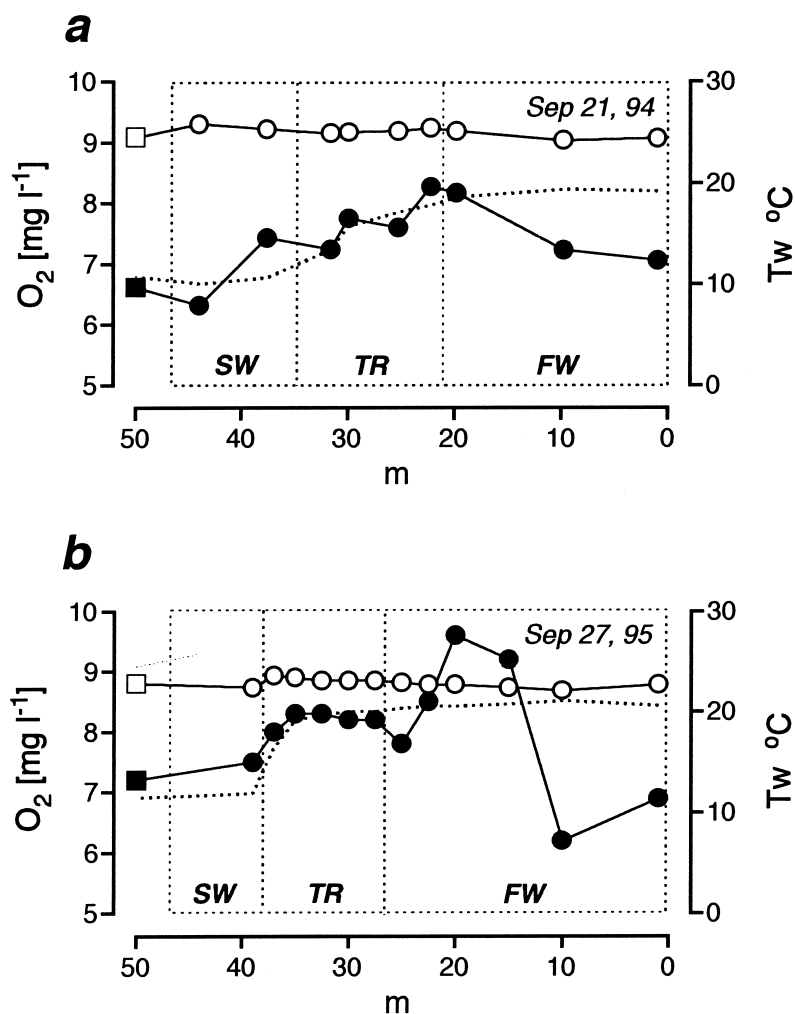


Figure 4. Dissolved O₂ concentration (closed symbol) and water temperature (open symbol) of beach ground water on a) September 21, 1994, and b) September 27, 1995. Circle and square symbols denote measurements for ground water and surface seawater, respectively. Dotted lines show the theoretical dissolved O₂ concentration under atmospheric equilibrium. Abbreviations are as follows: SW-sea water zone, TR-transition zone, FW-freshwater zone.

The observed NO₃⁻ decrease toward the shoreline (Figure 5) could be attributed to the dilution of freshwater NO₃⁻ by the seawater as well as to biological consumption of NO₃⁻. If there were no production and consumption of NO₃⁻, the NO₃⁻ concentration in ground water could be calculated by regarding Cl⁻ as a conservative tracer for seawater-freshwater mixing (Table 1). The observed NO₃⁻ concentrations were plotted against those calculated according to the mixing model (Figure 6a). All the data points, except for those of the end-members, are located below the 1:1

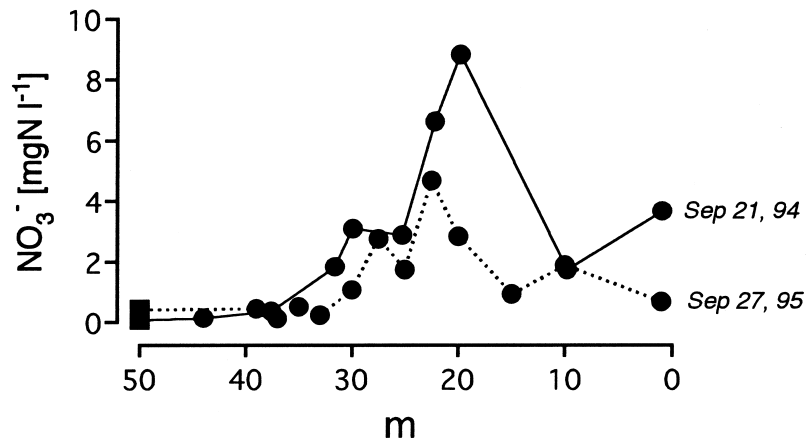


Figure 5. Nitrate concentrations in ground water [●] and seawater [■] on September 21, 1994 (solid line) and on September 27, 1995 (dotted line).

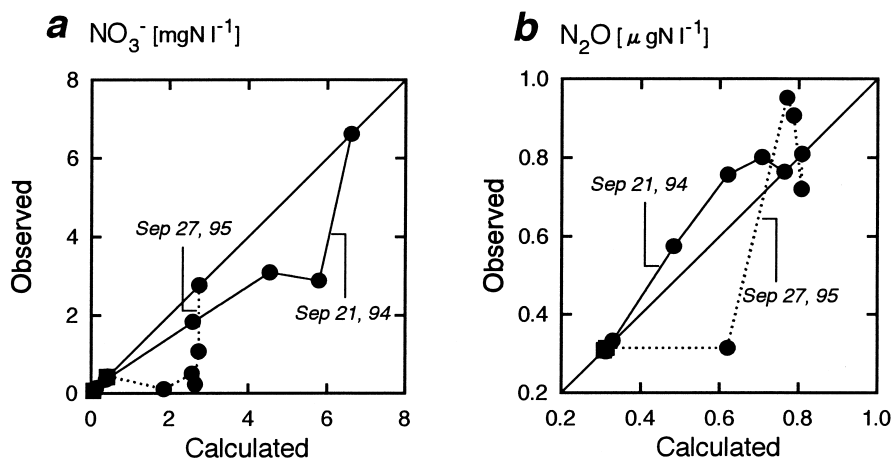


Figure 6. Comparison between measured and calculated concentrations of a) NO_3^- and b) N_2O . Solid circles and squares denote ground and seawaters, respectively. See text for explanation of the mixing model.

line in the diagram. This shows that other mechanisms, in addition to the mixing, cause the decrease in NO_3^- concentrations from TR-Z to SW-Z. The assimilation of NO_3^- by the very poor foliage of the sandy beach was of little importance with respect to the NO_3^- loss. Therefore, microbial assimilation and respiration of nitrate are probable sinks for the ground water NO_3^- .

The N_2O dynamics in ground waters would be useful for predicting the transformation of inorganic nitrogen in TR-Z because N_2O is an obligate intermediate of denitrification (Knowles 1982) and a by-product of ammonium oxidation (Hynes and Knowles 1984). The N_2O concentrations in the beach ground water exceeded those under atmospheric equilibrium conditions (130–800% saturation). The theo-

Table 1. Data used for two-source mixing model calculation.

		IN ¹⁾	OUT ²⁾
September 21, 1994	Cl ⁻ [g l ⁻¹]	1.18	17.2
	NO ₃ ⁻ [g N l ⁻¹]	6.62	0.07
September 27, 1995	Cl ⁻ [g l ⁻¹]	0.32	17.8
	NO ₃ ⁻ [g N l ⁻¹]	2.76	0.42

¹⁾ Ground waters at 22.0 m on September 21, 1994 and at 27.5 m on September 27, 1995.

²⁾ Surface seawater.

retical N₂O concentrations expected from the mixing of freshwater and seawater were calculated in the same manner as for NO₃⁻ and compared with the actual N₂O concentrations. The measured N₂O concentrations tended to exceed the calculated ones with a few exceptions (Figure 6b), which contradicted the NO₃⁻ trend. This indicates N₂O production in TR-Z, which is highly probable because the above calculation ignores N₂O emission from the ground water to the atmosphere. Ammonium oxidation was probably a minor source for N₂O in the beach ground water because of low NH₄⁺ concentrations in ground water (< 0.02 mg N l⁻¹). The NO₃⁻ consumption accompanied by the N₂O generation likely suggests the occurrence of denitrification in TR-Z, while microbial NO₃⁻ assimilation is an additional NO₃⁻ sink to denitrification.

The mixing model presented above might be criticized on the grounds that NO₃⁻ and N₂O concentrations in the ground water might vary not only horizontally but also vertically. Figure 7 compares the salinity and NO₃⁻ concentrations of samples obtained by the single-strainer wells with those from the multiple-strainer well. At the 34 m location, the salinity and the NO₃⁻ concentration fluctuated vertically. Their profiles resembled each other to give a fairly constant NO₃⁻/salinity ratio. The mean values for salinity and NO₃⁻ concentration of the vertical samples (multiple-strainer well) were comparable to the respective measurements for the vertically composite sample (single-strainer well). At the 25 m point, on the contrary, the NO₃⁻/salinity ratio changed vertically due to variation in NO₃⁻ concentrations (2.8–5.2 mg N l⁻¹). The mean NO₃⁻ concentration for the samples collected from different depths (3.8 mg N l⁻¹) was higher than the NO₃⁻ concentration of the vertically composite sample (3.0 mg N l⁻¹). Likewise, the N₂O concentrations might change with depth. The vertical determination of NO₃⁻ and N₂O concentrations in ground waters would be needed to get a realistic NO₃⁻ budget in the beach aquifer.

Denitrification potential

Distributions of nitrogenous compounds in the beach ground water indicated possible denitrification in TR-Z, although an accurate NO₃⁻ budget was hardly obtained due to the limitation of the sampling technique. Hence, the possibility of denitrification was examined from a microbial viewpoint. The potential activities of denitrification of the water-saturated sediments at 10 through 40 m varied from 1 to 40

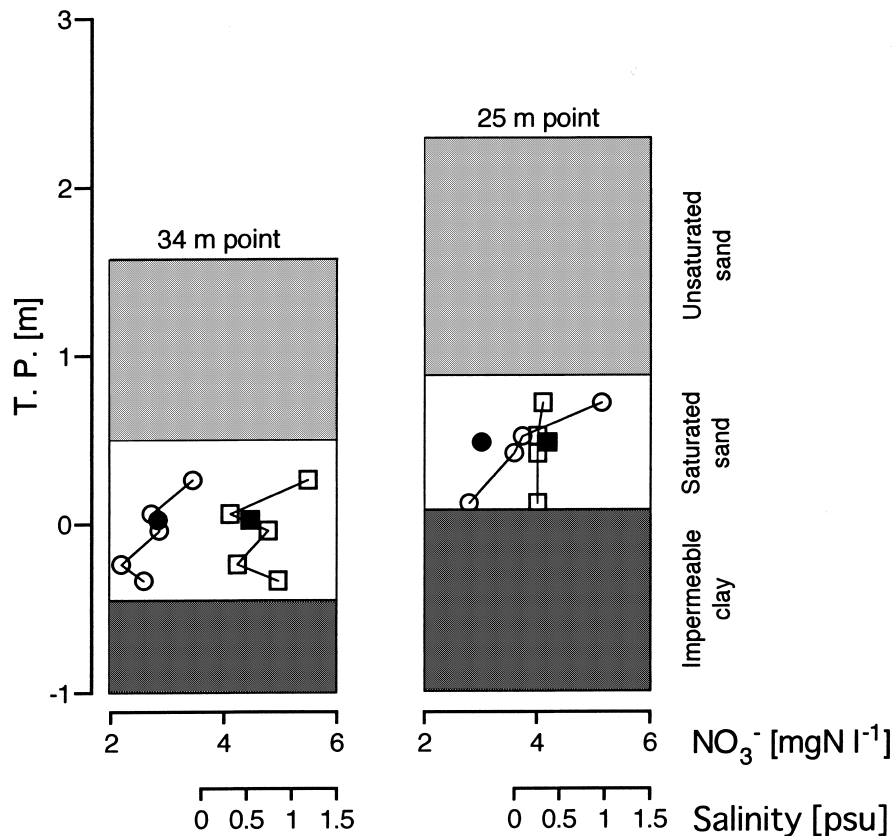


Figure 7. Vertical profiles of salinity [□] and NO_3^- concentration [○] obtained using the multiple-strainer well together with salinity [■] and NO_3^- concentration [●] in samples collected by the single-strainer wells. Samplings were carried out on August 25, 1995. Salinity is given as practical salinity unit (psu) that is calculated from electric conductivity and temperature of water.

$\mu\text{g N kg}^{-1} \text{ d}^{-1}$ (Figure 8a) and were comparable with those reported for the sandy aquifer sediments at Falmouth, Massachusetts ($4\text{--}82 \mu\text{g N kg}^{-1} \text{ d}^{-1}$; Smith and Duff (1988) and Smith et al. (1991)). Potential denitrification activity tended to be maximal at around 25 m, in the middle of the observation line. Concentrations of Cl^- in the ground water were in the $0.1\text{--}0.3 \text{ g l}^{-1}$ range at the point of maximum denitrification potential. The MPN of denitrifiers was largest at the most landward point and lowest in the vicinity of the shoreline (Figure 8b). Site-to-site variation in the MPN of denitrifiers with a maximum at 25 m resembled that of potential denitrification activity. These microbial data confirm that denitrification can take place in the beach sediments under certain conditions, such as in anaerobic micro-sites.

Our incubation methods did not adjust the salinity of media to the *in situ* conditions. Fluctuation in osmotic pressure might have affected bacterial physiology and could have biased the measurements for the activity and MPN of denitrifiers.

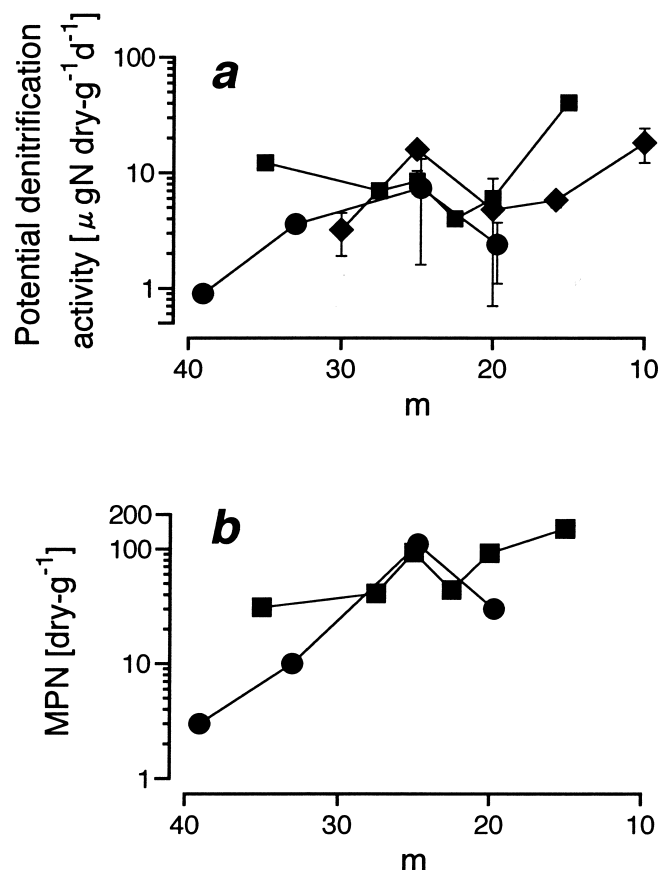


Figure 8. Potential denitrification activity (a), and most probable number of denitrifiers (b) on July 11, 1995 [●], September 26, 1995 [■], and July 3, 1996 [◆]. The MPN was not determined with the samples collected on July 3, 1996.

For the activity measurement, the stress must have been most pronounced for samples in SW-Z. For sediments at 40 m (water content of 20% in weight), salinity in the vial was two-thirds of that *in situ* after addition of KNO_3 solution. The salinity effect on activity measurements for other sediment samples was not serious because bacteria were also exposed to salinity fluctuations under field conditions as a result of migration of the low chlorinity region caused by on-site precipitation recharge. In fact, Cl^- concentrations at 25 m varied from 0.05 g l^{-1} on July 12, 1995 to 3.18 g l^{-1} on September 27, 1994 (Figure 2b). Concerning the MPN enumeration, salinity was constant when the inoculum was prepared using physiological salt solution. In this case, samples with *in situ* salinity being higher or lower than the physiological salt solution might result in underestimation of MPN. Contrary to this expectation, the sediment at 15 m whose *in situ* salinity was the lowest among all incubated sediments gave the largest MPN (Figure 7b). This suggests

that the observed spatial variations in the potential activity and MPN of denitrifiers reflect the true distributions, despite the salinity differences between field and incubation.

Mechanisms sustaining the maximum zone of denitrification potential

The small spatial variations of temperature and the degree of aeration of the ground water hardly identified a maximum-denitrification activity zone (DN-Z) in the slightly saline region. This meant in turn that denitrification activity was subject to the availability of substrate, electron acceptors (nitrogen oxides) and electron donors (organic matter). As NO_2^- concentrations were low in ground waters ($< 0.02 \text{ mg N l}^{-1}$), NO_3^- was the overwhelming electron acceptor for denitrification. Concentration of NO_3^- in beach ground water increased at around the DN-Z (Figure 5), which was probably due to an additional source of NO_3^- such as the oxidation of nitrogen compounds in wastewater. Thus, the NO_3^- availability was probably the dominant control for the location of DN-Z.

Distribution of organic matter (OM) in the beach sediments is also plausible to determine the DN-Z position. Organic carbon and nitrogen contents in the fine sediment tended to decrease toward the seashore, with a notable maximum in DN-Z (Figure 9a, 9b). A similar tendency was observed for the C/N atomic ratio (Figure 9c). These results suggest a concurrent change in the amount and in the chemical characteristics of OM within DN-Z. The $\delta^{13}\text{C}$ variation of the OM in the fine sediments of the beach (Figure 9d) was a mirror image of the C/N ratio (Figure 9c). The $\delta^{13}\text{C}$ was inversely related to the C/N ratio (Figure 10, F-test $p < 0.01$). A correlation between the C/N ratio and $\delta^{13}\text{C}$ was also found with particulate organic matter (POM) in estuarine environments (Tan and Strain 1979; Matson and Brinston 1990; Ogawa et al. 1994). Since two-component mixing models have successfully elucidated the land and marine production as sources for the estuarine POM, a similar model was tested to elucidate the origins of the OM in the fine sediments of the studied beach. The $\delta^{13}\text{C}$ of POM in marine origin was calculated as -16.5‰ by substituting the mean C/N ratio of phytoplankton biomass (6.6; Redfield (1958)) in the regression equation in Figure 10. This value falls between two mean values for $\delta^{13}\text{C}$ of POM in Tokyo Bay in different periods, -17.2‰ (Ogura et al. 1986) and -15.6‰ (Ogawa et al. 1994). These $\delta^{13}\text{C}$ values of POM are considerably higher than the values expected under CO_2 -rich conditions around -20‰ (Degens et al. 1986). The primary products are typically enriched in ^{13}C when isotopically lighter CO_2 becomes depleted in water due to accelerated photosynthesis under nutrient-rich conditions. POM with $\delta^{13}\text{C}$ values higher than -20‰ was observed in eutrophic environments, such as the Delaware estuary (Cifuentes et al. 1988), the estuaries of the Pamlico and Newse Rivers (Matson and Brinston 1990), and Mikawa Bay (Takahashi et al. 1992). These facts suggest the primary production in Tokyo Bay to be one of the two ultimate sources for the OM in fine sediments on the beach. Terrestrial production is probably the other end-member of the mixing model. $\delta^{13}\text{C}$ values between -28 and -26‰ were reported for riverine POM influxing Tokyo Bay (Ogura et al. 1986; Ogawa and Ogura 1997). By substituting

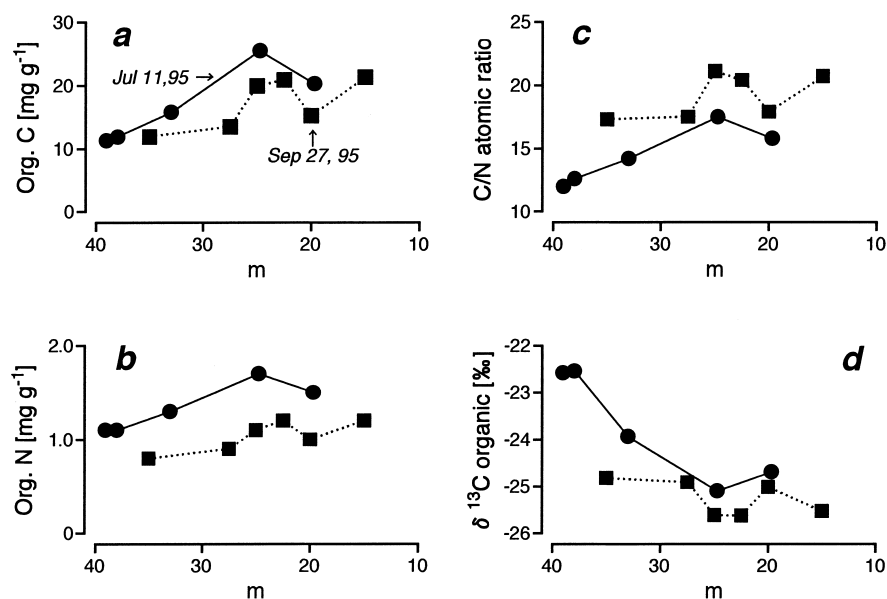


Figure 9. Organic carbon content (a), organic nitrogen content (b), C/N atomic ratio (c), and $\delta^{13}\text{C}$ values of organic carbon (d) of fine sediment collected on July 11 [●] and September 26 [■], 1995.

these $\delta^{13}\text{C}$ values in the regression equation, the C/N ratios of the terrestrial POM becomes 45 to 22. This range is reasonable for terrestrial organic matter in a temperate climate region (e.g., 25–400; Hedges et al. (1986)). Nitrogen-rich POM discharged from Tokyo Metropolitan may lower the C/N ratio of the river POM to some extent. From the isotopic results, organic matters derived from land and marine primary productions were the two ultimate sources of the OM in the fine sediments of the beach. The contribution of the terrestrial fraction to the OM varied horizontally from 58% to 92% in the beach sediment. The contributions of terrestrial origin may be underestimated because C/N ratios decrease and $\delta^{13}\text{C}$ values increase during decomposition.

On September 27, 1995, the OM at 35 m showed a high C/N ratio (Figure 9c) and a low $\delta^{13}\text{C}$ value (Figure 9d). Both denitrification potential and MPN were elevated at this point (Figure 8a, 8b). This indicates that the denitrification potential increases where the OM is rich in the fraction of terrestrial origin because terrestrial OM is typically characterized by higher C/N ratios and lower $\delta^{13}\text{C}$ values than marine OM (e.g., Fry and Sherr (1984)). Murphy et al. (1989) reported high C/N ratios (99–131) and low $\delta^{13}\text{C}$ values (–24 to –27‰) for dissolved organic matter (DOM) in the Milk River aquifer, Alberta, Canada. Schiff et al. (1990) also found DOM with low $\delta^{13}\text{C}$ values in Ontario. Assuming a high C/N ratio and a low $\delta^{13}\text{C}$ value for DOM in the discharging ground water, immobilization or deposition of the DOM due to a salinity change can explain the increase of the C/N ratio and the decrease of the $\delta^{13}\text{C}$ value in the OM in DN-Z. However, unfortu-

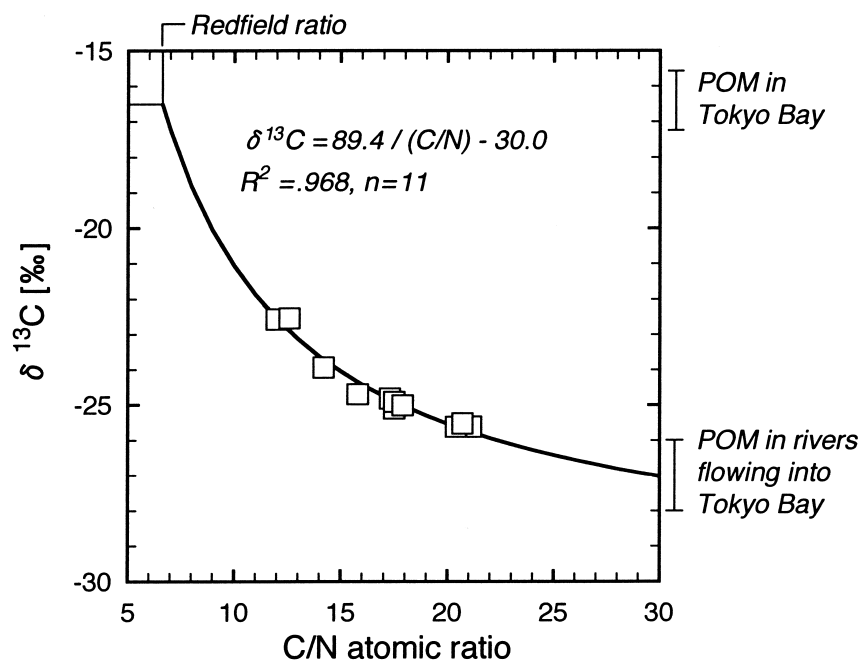


Figure 10. Relationship between C/N atomic ratio and the $\delta^{13}\text{C}$ value of POM.

nately, the present study did not determine either the C/N ratio or the $\delta^{13}\text{C}$ value of DOM in ground water.

Conclusion

The potential activity of denitrification in the water-saturated sediments of a sandy beach was highest where fresh ground water began to mix with seawater. Field observations demonstrated that net NO_3^- consumption and net N_2O production occurred in the region of high denitrification potential. The discharge of fresh ground water continuously supplied NO_3^- to denitrifiers in the beach aquifer. Energy source availability increased in slightly saline sediments, possibly through precipitation of DOM of terrestrial origin. The boundary zone between FW-Z and TR-Z fulfilled the demands of denitrifiers for electron donors and acceptors simultaneously. As the beach ground waters were rich in dissolved O_2 , the denitrification probably occurred in anaerobic micro-sites of the sediment. Most denitrifying bacteria could use O_2 as a terminal electron acceptor. From the viewpoint of organic matter availability, the DN-Z may be a suitable habitat, not only for denitrifiers but also for other heterotrophic microbes.

A certain fraction of ground water NO_3^- might be fixed as a microbial biomass in the studied beach. In order to evaluate the relative importance of denitrification and assimilation in NO_3^- consumption, precious NO_3^- budget is inevitable, which

could not be obtained by this study because of the lack of an accurate NO_3^- profile in beach ground water. While further studies are needed for a quantitative discussion, it is possible to conclude that partial NO_3^- discharged to a natural sandy beach is microbially transformed before entering the sea.

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